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PPARS, METABOLIC DISEASE AND ATHEROSCLEROSIS

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PPAR-alpha belongs to the family of nuclear receptors. Activated PPAR-alpha stimulates the expression of genes involved in fatty acid and lipoprotein metabolism. PPAR-alpha activators, such as the normolipidaemic fibric acids, decrease triglyceride concentrations by increasing the expression of lipoprotein lipase and decreasing apo C-III concentration.

Furthermore, they increase HDL-cholesterol by increasing the expression of apo A-I and apo A-II. PPAR-alpha activation by fibric acids improves insulin sensibility, and decreases thrombosis and vascular inflammation. PPAR-alpha activators (gemfibrozil) decrease the risk of coronary heart disease in patients with normal LDL-cholesterol and low HDL-cholesterol (VA-HIT) and they slow the progression of premature coronary atherosclerosis (BECAIT) (bezafibrate), particularly in patients with type 2 diabetes (DAIS) (fenofibrate).

KEY WORDS: PPAR-alpha, lipoprotein metabolism, atherosclerosis, metabolic disease.

INTRODUCTION

Epidemiological and intervention studies have now confirmed that dyslipidaemias are major risk factors for atherosclerosis and coronary artery disease (CAD). Primary [1] and secondary [2] intervention trials with HMG-CoA reductase inhibitors have undoubtedly proved that a drastic reduction in LDL-cholesterol levels reduces the cardiovascular risk in hyper-LDL-cholesterolaemic patients and even in patients considered as normo-LDL-cholesterolaemics [3]. Nevertheless, other dyslipidaemias, such as hypoalphalipoproteinaemia (low plasma HDL) associated, or not, with concomitant hypertriglyceridaemia, may be the cause of a substantial number of cases of CAD [4, 5].

In the clinic, PPAR-alpha activators are chemically related to fibric acids (clofibrate, gemfibrozil, fenofibrate, bezafibrate and ciprofibrate).

Fibrates are used in the treatment of hypertriglyceridaemia with or without hypoalphalipoproteinaemia [3, 4] and, recently, the VA-HIT (Veterans Affairs-High Density Lipoprotein Cholesterol Intervention Trial) study with a median follow-up of 5.1 years [6] clearly demonstrated that raising HDL-cholesterol and lowering triglycerides, without lowering LDL-cholesterol with gemfibrozil, in men with documented CAD and low HDL-cholesterol reduced the incidence of death from CAD and of non-fatal myocardial infarction by 22% without reducing total mortality.

Nevertheless, although fibrates have been used in clinical practice for over three decades now, in-depth knowledge of the molecular mechanism of their normolipidaemic effects remained a mystery.

Recently, a direct relationship was evoked between PPAR-alpha activation by fibrates and alteration in lipoprotein metabolism. Furthermore, *in vivo* experiments in animals and *in vitro* studies suggest that, in humans, fibrates might not only reduce atherosclerosis development through their normolipidaemic properties but also by reducing inflammation at the level of the vascular wall and thrombosis. In this paper we review the current knowledge on the role of PPAR-alpha in metabolic diseases and atherosclerosis.

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARS)

PPARs belong to the family of hormonal activated nuclear receptors. Activated PPARs heterodimerize with activated Rexinoid-X-Receptor (RXR) and bind to the specific so-called 'Peroxisome Proliferator Response Elements' (PPREs) which are localized in the promoter of target genes. PPRE(s) are constituted of direct repeat (DR) hexameric sequences which are separated

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by one or two nucleotides (DR1, DR2). The binding of PPAR to the PPRE induces the expression of the target gene.

To date, three different sub-types of PPARs have been reported (alpha, delta, gamma); each specific PPAR sub-type is encoded by one specific gene.

PPAR-alpha is highly expressed in liver, heart, kidney and in brown adipose tissue and moderately in bowel, skeletal muscle, thymus and testis [7].

PPAR-ALPHA ACTIVATORS

PPAR-alpha activators have been synthesized. They include fibric acid derivations (fibrates) and they all induce liver peroxisome proliferation, hepatomegaly and liver cancer in rodents [8].

FIBRIC ACIDS

Wy-14 643 and fibric acids (clofibrate, ciprofibrate, bezafibrate, fenofibrate, gemfibrozil...) were developed as hypolipidaemic agents in rodents. Clofibric acid and fenofibric acid activate both PPAR-alpha and PPAR-gamma with a 10-fold selectivity for PPAR-alpha. Some fibric acids such as bezafibrate have no specificity for any of the three sub-types of PPARs. One common outstanding pharmacological property of clinically used fibric acids is their low affinity for PPAR-alpha [EC50 (μM)] and the resulting required oral high doses (300–1200 mg/day) to achieve clinical efficiency. Newly synthesized PPAR-alpha activators with more than 1000-fold affinity for PPAR-alpha might be promising new drugs for the treatment of dyslipoproteinaemia [9].

EFFECTS OF FIBRIC ACIDS ON PLASMA LIPIDS

Fibric acids are first-line drugs in the treatment of primary hypertriglyceridaemia and are very useful in the treatment of combined hyperlipidaemia, type III dyslipoproteinaemia and secondary lipid abnormalities observed in Non-Insulin Dependant Diabetes Mellitus (NIDDM) and obese individuals.

PPAR-alpha activation by fibrates leads to:

— decreased hypertriglyceridaemia by increasing LPL expression [10] and decreasing apo C-III expression [11];

— increased HDL-cholesterol, apo A-I and apo A-II levels [12–14] in human plasma at least partly by increasing apo A-I and apo A-II expression [13, 14];

— reduced LDL-cholesterol in combined hyperlipidaemia [15] by decreasing the levels of atherogenic dense LDL, which have poor affinity to the LDL receptor, while increasing buoyant LDL which displays high affinity to this receptor. In primary hypercholesterolaemia, fibrates reduce dense LDL, but not light LDL fractions [16]. In hypercholesterolaemic patients, Caslake *et al.* [17] observed that fenofibrate significantly decreased LDL-cholesterol (30%) without decreasing LDL apo B production by shifting LDL from a slowly catabolized pool towards a rapidly catabolized one. Furthermore, the rate of apo B-LDL degradation by the receptor route rose 43% with the drug, whereas the amount cleared by the receptor-independent pathway did not change.

It is generally acknowledged that therapeutic concentrations of fibrates do not inhibit HMG-CoA reductase activity [18].

EFFECT OF FIBRATES ON LIPOPROTEIN METABOLISM

Recent studies have shown that the effects of fibrates on lipoprotein metabolism are due to an increase in cellular FFA catabolism and the resulting inhibition of hepatic VLDL triglyceride secretion, as well as to alterations in genes governing the intravascular hydrolysis of triglycerides and those governing HDL production.

Effects of fibrates on FFA metabolism

PPAR-alpha is highly expressed in tissues with elevated rates of FA catabolism, where it regulates genes involved in FA uptake, activation into acyl-CoA esters, degradation via the peroxisomal and mitochondrial beta-oxidation pathways and ketone body synthesis [19].

Fibrate treatment is known to activate PPAR-alpha induced FATP mRNA levels in rat liver and intestine and ACS mRNA levels in the liver and kidney.

PPAR-alpha regulates the entry of FAs into the mitochondria, which is a crucial step in their metabolism, especially in tissues like heart, skeletal muscle and brown adipose tissue in which FAs are a major source of energy.

Three distinct uncoupling protein isoforms, UCP-1, UCP-2 and UCP-3 have been identified and implicated as mediators of thermogenesis. Kelly *et al.* [20] reported that the treatment of rats or db/db mice with WY-14 643 (PPAR-alpha ligand) did not affect the expression of UCP-1, 2 or 3 in brown adipose tissue. Nevertheless, hepatic UCP-2 mRNA was increased (×4 over the control level) in db/db and lean mice, although this effect was not observed in rats. This data shows that PPAR-alpha activators may also regulate UCP proteins, which may be an end-step in the FA catabolic actions of these drugs.

This data as a whole shows that PPAR-alpha activators stimulate different steps in FA oxidative metabolism in different organs and particularly in the liver where they reduce the quantity of FA available for VLDL synthesis and secretion.

Effects of fibrates on genes involved in lipoprotein metabolism

Triglyceride-rich lipoprotein metabolism. One of the major effects of PPAR-alpha activators on plasma lipid metabolism is to reduce triglyceride levels. Kesaniemi

et al. [21] showed that gemfibrozil decreased the production of VLDL triglyceride by an average of 28%.

Nevertheless, as shown by Kesaniemi *et al.* [21], inhibition of VLDL triglyceride synthesis is not the unique hypotriglyceridaemic effect of fibrates. In this study, gemfibrozil reduced VLDL triglyceride synthesis by only 28% but increased the fractional catabolic rate of VLDL triglyceride by 92%. This suggests that PPAR-alpha activators decrease plasma triglyceride concentrations by increasing VLDL- and chylomicron-triglyceride hydrolysis.

In fact, fibrates increase post-heparin plasma LPL activity.

Schoonjans *et al.* [22] demonstrated that inducibility of the LPL gene by PPAR-alpha correlated with the tissue distribution of this nuclear receptor in rat.

A sequence element was identified as a PPRE in the human LPL promoter that mediates the functional responsiveness to PPAR-alpha activators. The main effect of fibrates is, therefore, on LPL production in rat liver.

Apo C-III acts by delaying the catabolism of triglyceride-rich particles by inhibiting their binding to the endothelial surface and lipolysis by LPL, as well, by interfering with apo E-mediated receptor clearance of remnant particles from plasma [23–28].

Using PPAR-alpha deficient mice, Peters *et al.* [29] demonstrated an obligatory role for PPAR-alpha in the repression of apo C-III gene expression by fibrates. The regulation of apo C-III gene transcription is complex, being governed by an ensemble of transcription factor binding sites within 1 Kb upstream of the transcription initiation site. Among these sites is the C3P (also called CIIIB) site, to which a number of nuclear receptors such as HNF-4, ARP-1, Ear/COUP-TF [30], RXR and PPAR-alpha bind [31]. Whereas HNF-4, RXR and PPAR-alpha [31] can activate apo C-III gene transcription via this site, ARP-1 and Ear3/COUP-TF act as repressors [30]. Further studies are required to determine whether apo C-III transcriptional repression by PPAR-alpha activators involves any or all of these nuclear factors.

In severe primary hypercholesterolaemia, fenofibrate therapy decreased apo C-III and lipoprotein particles containing both apo C-III and apo B [32]. Staels *et al.* [11] demonstrated that fibrates down-regulate apo C-III expression independently of any induction of peroxisomal acyl CoA oxidase. These studies show that PPAR-alpha activators decrease human and rat liver apo C-III expression, but the molecular mechanism of this down-regulation has not yet been fully elucidated.

HDL metabolism. Fibric acid therapy increases HDL-cholesterol plasma levels (≈ 10–15%) in hypertriglyceridaemia [33], combined hyperlipidaemia [34, 35] and hypercholesterolaemia [35, 36]. These increases in HDL-cholesterol levels are associated with significant increases in levels of apo A-I and apo A-II. Malmendier *et al.* [12] showed that fenofibrate increased apo A-I in hypercholesterolaemic patients by increasing its synthetic rate much more than its catabolic rate.

Recent studies have demonstrated, in humans, that fibric acids increase plasma HDL concentrations, at least in part, through the induction of the expression of the human apo A-I and apo A-II genes [13, 14, 37].

Vu-Dac *et al.* [13] showed that the transcription rate of the human apo A-I gene is induced by PPAR-alpha which interacts with a positive PPRE located in the A site of the human apo A-I gene promoter liver specific enhancer.

In 1995, Vu Dac *et al.* [14] reported that fibric acids induced apo A-II mRNA in primary cultures of human hepatocytes and in human hepatoblastoma cells resulting in increased apo A-II secretion in both cell culture systems. These authors identified a DR1-type PPRE in the J-site of the human apo A-II promoter and demonstrated that fibric acids increase apo A-II plasma levels by stimulating transcription of its gene through the interaction of activated PPAR-alpha with the apo AII-PPRE.

Recently, it has been reported that fibrates increase HDL-receptor activity in human macrophages by stimulating the expression of SR-BI/CLA-1 [38] and ABCA1 [39]. These two receptors have been shown to be capable of binding HDL to plasma membrane and of inducing free cholesterol efflux from foam cells derived from human macrophages. This cellular cholesterol efflux corresponds to the first step in the so-called 'reverse cholesterol transport' which is responsible for returning excess peripherical cholesterol to the liver to eliminate it in biliary secretion. Therefore, fibrates would not only increase reverse cholesterol transport through increasing the number of cholesterol carriers (HDL) but they would also increase the cellular expression of the HDL receptors whose task is to ensure the binding of these carriers to cell membrane and to induce the efflux of excess cellular cholesterol.

EFFECT OF FIBRIC ACIDS ON INSULIN SENSIBILITY AND ADIPOSITY

Until recently, the effect of PPAR-alpha activators on insulin sensitivity were not clearly demonstrated. Therefore, we decided to test the effects of PPAR-alpha activators on insulin sensibility in insulin resistant rats. Fenofibrate, ciprofibrate and GW9578 were tested in two rodent models of high fat diet induced (C57BL6/mice) or genetic (obese Zucker rats) insulin resistance [40]. These compounds markedly lowered hyperinsulinaemia and, when present, hyperglycaemia in both animal. These drugs improved insulin action or glucose utilization. In addition, fenofibrate treatment prevents high fat diet-induced increase of body weight and adipose tissue mass without influencing calorie intake. This data suggests that selective PPAR-alpha activators reduce insulin resistance without any significant effects on body weight and adipose tissue mass in rodent models of insulin resistance.

Plasma non-esterified fatty acids are increased in subjects with type 2 diabetes suggesting that PPAR-alpha could link this metabolic disease and dyslipidaemia, and affect response to fibrates. Two polymorphisms were detected in PPAR-alpha gene, one in intron 3 and a missense mutation, leucine 162 to valine, in the DNA binding domain [41]. In type 2 diabetics, V162 allele carriers had higher apo A-I concentrations. By contrast, no effect was observed in healthy rare allele carriers. *In vitro*, the V162 variant showed greater transactivation of a reporter gene construct. This study shows that naturally occurring variation alter PPAR-alpha function, influencing plasma lipid concentrations in type 2 diabetes but not in healthy people. This demonstrated that PPAR-alpha is a link between diabetes and dyslipidaemia, and so could influence the risk of coronary artery disease. In another recent report [42] association studies were undertaken in two populations of type 2 diabetic patients from Pondichery and from France [L162V (exon 5) and A268V (exon 7) polymorphisms]. No association was found between these polymorphisms and diabetes or coronary heart disease. However, in the Caucasian diabetic male population with coronary heart disease, the Val 162 allele carriers showed higher concentrations of total cholesterol and apo B when compared to non-carriers. A trend toward elevated concentrations of total cholesterol and apo B was also observed in the Caucasian diabetic male patients without coronary heart disease carrying Val 162 allele. Therefore, it is likely that the PPAR-alpha gene does not have a major role in diabetes and CHD in these populations, although a minor contribution of the PPAR-alpha gene to the risk of coronary heart disease associated with type 2 diabetes cannot be excluded through a modulation of atherogenic plasma lipids.

EFFECT OF FIBRIC ACIDS ON THROMBOSIS

Acute coronary artery disease depends on the activation of the different factors of the pro-thrombotic cascade and/or of the inhibition of the anti-thrombotic factors. Fibric acids decrease PAI-1 production in cultured cynomolgus hepatocytes [43] but there is no correlation between the inhibition of PAI-1 production and the PPAR-alpha transactivation activity. In humans, gemfibrozil and bezafibrate increased plasma PAI-1 activity [44] but ciprofibrate [45] did not modify its plasma levels.

Fibric acids influence plasma fibrinogen levels. Gemfibrozil increased plasma fibrinogen levels [45], while bezafibrate [45] and fenofibrate [35] significantly decreased this concentration and ciprofibrate had no effect [45]. Nevertheless, ciprofibrate undoubtedly decreased the functional fibrinogen activity while the observed effects of gemfibrozil on this parameter depended on the analytic method applied [45]. Kockx *et al.* [46] showed that *in vivo* (in mice), fibric acids, decreased hepatic fibrinogen alpha-, beta- and gamma-chain mRNA levels.

We have recently demonstrated that fibric acids inhibit Tissue Factor expression in human monocytes and macrophages [47]. This data suggests that fibric acids might have an important role in preventing an early step of thrombotic cascade.

EFFECT OF FIBRIC ACIDS ON VASCULAR INFLAMMATION

Atherosclerosis development is a long-term process which involves the recruitment and the activation of different cells such as macrophages, T-lymphocytes, smooth muscle cells and endothelial cells which elicits a local inflammatory response [48].

PPAR-alpha is expressed in atherosclerotic plaques and in primary cultures of endothelial cells [49], smooth muscle cells [50] and macrophages [51]. Chinetti *et al.* [51] showed that PPAR-alpha is expressed in differentiated human monocyte-derived macrophages and already present in undifferentiated monocytes. They showed that PPAR-alpha ligands induce apoptosis of macrophages activated with tumour necrosis factor-α and interferon-γ.

As early as 1996, Devchand *et al.* [52] reported that leukotriene B4 (LTB4), a pro-inflammatory molecule, is an activating ligand for PPAR-alpha which is involved in the regulation of the oxidative degradation of FAs and their derivatives, amongst which is LTB4 itself. Therefore, the pro-inflammatory effect of LTB4 might be counteracted by the stimulation of its own degradation through its PPAR-alpha activation, indicating an anti-inflammatory role for PPAR-alpha. Recent data has shown that WY-14 643 inhibits Inducible NO synthase (iNOS), a key inflammatory enzyme, in macrophages [53].

To determine whether PPAR-alpha interferes with the response of human aortic SMC to inflammatory cytokines, Staels *et al.* [54] analysed the influence of fenofibric acid on IL-1-mediated activation of IL-6 production, a marker for SMC activation. Fenofibric acid prevented the IL-1-induced secretion of IL-6 in a dose-dependent manner. This inhibition occurs at fenofibric acid concentrations required for the induction of positive PPAR-alpha response genes and within the range of plasma concentrations found in humans. Incubation of smooth muscle cells with IL-1 increases 6-keto-prostaglandin F1α (6-keto-$PGF_{1\alpha}$) secretion 10-fold. Fenofibric acid treatment prevented the formation of 6-keto-$PGF_{1\alpha}$ by preventing COX-2 induction by IL-1 as a result of a negative regulation of COX-2 transcription through a negative regulation of NF-kappa B transcription activity.

Delerive *et al.* [55] recently showed that negative interference of PPAR-alpha with AP-1 signalling explains the repression of thrombin-induced endothelin-1 (ET-1)

expression in endothelial cells by PPAR-alpha ligands. This study suggests that PPAR-alpha activators might reduce coronary events by reducing vasospasm and atherosclerosis development following fibric acid treatments. Recently, they confirmed [55] that activation of PPAR-alpha by fibric acids negatively regulates the vascular inflammatory gene response by negative cross-talk of activated PPAR-alpha with transcription factors NF-Kappa B and AP-1 (protein–protein interaction with p65 and c-jun).

EFFECTS OF PPAR-ALPHA ACTIVATORS IN THE TREATMENT OF DYSLIPOPROTEINAEMIAS AND IN THE PREVENTION OF ATHEROSCLEROSIS

Reduction of triglyceride and/or increase in HDL-cholesterol plasma levels

In order to stress the primary targets of fibrates (high triglycerides and low HDL-cholesterol plasma levels) we will present recent clinical data with fibrates that induce strong reduction of triglyceride plasma levels (gemfibrozil, bezafibrate).

Gemfibrozil. In 1997, data of the LOCAT's study (Lipid Coronary Angiography Trial) [56] showed that gemfibrozil therapy retarded the progression of coronary atherosclerosis and the formation of bypass-graft lesions after coronary bypass surgery in men with low HDL cholesterol as their main lipid abnormality.

Syvänne *et al.* [57] have studied which lipoproteins, separated by preparative ultracentrifugation, predict angiographic progression in this population. Analysis of the lipoprotein compositions clearly showed that all lipoprotein classes were significantly depleted of triglycerides by gemfibrozil. VLDL were both decreased in number and depleted of lipid, but there was no suggestion of any reduction of IDL or increase of HDL2 particle numbers. Total serum cholesterol and both triglyceride and cholesterol in the IDL and LDL fractions were positively and significantly associated with the risk of global angiographic progression and HDL cholesterol concentration was not associated with protection against progression.

This paper adds to the growing evidence of the atherogenecity of triglyceride-rich lipoproteins, especially IDL, and the antiatherogenic influence of HDL3 and suggests that reductions of triglyceride levels that are commonly considered normal seem to provide protection against progressive CAD.

The objective of the Veterans Affairs-High Density Lipoprotein Cholesterol Intervention Trial (VA-HIT) [6] was to test if gemfibrozil decreases CAD death and non-fatal myocardial infarctions in men with documented CAD and HDL-cholesterol $\leq$ 40 mg dl^{-1}, LDL-cholesterol $\leq$ 140 mg dl^{-1} and triglycerides $\leq$ 300 mg dl^{-1}. 2531 patients enrolled into the study and the median follow-up was 5.1 years.

Gemfibrozil (1200 mg/day) decreased total cholesterol by 2.8% and triglycerides by 24.5% but had no effect on LDL-cholesterol and increased HDL-cholesterol by 7.5%.

Gemfibrozil treatment reduced coronary heart death [by 22% (P = 0.006)] and non-myocardial infarction [274 (21.6%) and 219 (17.3%) in the placebo and gemfibrozil group, respectively]. Furthermore, stroke was less frequent in the gemfibrozil group but there was no difference in the rates of coronary revascularization, or hospitalization due to unstable angina between the two groups, as there was no difference in the total mortability between the two groups nor in the frequency of new malignancies.

Therefore VA-HIT provides direct clinical evidence of a beneficial effect of reducing triglycerides and increasing HDL-cholesterol without affecting LDL-cholesterol in secondary prevention in patients with low HDL-cholesterol and low-cholesterol.

Bezafibrate. The Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT) was initiated to determine whether bezafibrate retards the progression or facilitates regression of premature coronary atherosclerosis [58–60]. The angiographic findings over the 5 years of study indicated that the median change in minimum lumen diameter (MLD) at final assessment was on average 0.13 mm less in the bezafibrate group than in the placebo group ($P < 0.049$).

In 1998, Ruotolo *et al.* [61] examined if there was a relationship between the progression of coronary lesions in the BECAIT and lipoproteins and lipoprotein sub-fractions. In addition to the decrease in VLDL-cholesterol (−53%) and triglyceride (−46%), bezafibrate treatment resulted in a significant increase in HDL3-cholesterol (+9%) and a shift in the LDL sub-class distribution toward larger particle species without any effect on LDL-cholesterol levels. Decreases in small dense LDL and/or VLDL lipid concentrations were unrelated to disease progression. These data suggest that the effect of bezafibrate on progression of focal coronary atherosclerosis could, at least partly, be attributed to a rise in HDL3-cholesterol and a decrease in the total number of apo B-containing lipoproteins.

The goal of the Bezafibrate Infarction Prevention (BIP) [62] was to test the benefit of a therapy that increases serum HDL-cholesterol concentrations and lowers triglyceride concentrations on the reduced incidence of myocardial infarctions and mortality among CAD patients

Bezafibrate treatment significantly reduced serum triglycerides (22%) but not serum total cholesterol (4%) nor LDL-cholesterol (5%), and significantly increased HDL-cholesterol (12%).

Bezafibrate treatment induced 0.13 mm less progression in coronary MLD [63], but did not significantly reduce the primary end point (fatal or non-fatal myocardial infarction plus sudden death) (−9%, P = 0.27) with

a median follow-up of 7 years [64] and did not modify total mortality ($P = 0.64$) (28, 31). Nevertheless, subgroup analysis suggested that bezafibrate had only a beneficial effect in patients with serum triglycerides above 2.3 mmol l^{-1} (200 mg dl^{-1}) ($P = 0.03$) where it significantly decreased primary end-point ($P = 0.03$).

Fenofibrate. The incidence of CAD is greatly increased in those with diabetes mellitus. The Diabetes Atherosclerosis Intervention Study (DAIS) [65] is the first intervention trial designed to examine directly whether correcting dyslipoproteinaemia in men and women with non-insulin-dependent diabetes will reduce their CAD. The DAIS is a multinational angiographic study using the 200 mg micronized form of fenofibrate in a double-blind, placebo-controlled protocol. Preliminary oral reports have indicated that fenofibrate reduced coronary stenosis progression in type 2 diabetes.

MIXED DYSLIPOPROTEINAEMIA

It is clearly demonstrated that the convenient treatments for pure hypercholesterolaemia and pure hypertriglyceridaemia are statins and fibrates, respectively. However, the most appropriate therapy of combined hyperlipidaemia remains to be determined. Zambon *et al.* [34] compared in a randomized crossover study the effects of gemfibrozil *vs* lovastatin in familial combined hyperlipidaemia and the additive effects of combination treatment on lipid regulation. Gemfibrozil (1200 mg/day) had no effect on LDL-cholesterol levels but favourably influenced triglyceride levels and apo B-containing lipoprotein composition that are related to hypertriglyceridaemia (reduction of both the number and size of VLDL particles). Conversely, lovastatin markedly decreased LDL-cholesterol (reduction of the number of LDL particles) but had little effect on triglyceride-rich lipoproteins. Combined treatment was safe and had additive effects on lipids, causing significant reduction in total cholesterol, triglycerides, LDL-cholesterol and an increase in HDL-cholesterol. In this condition, target LDL-cholesterol levels (<130 mg dl^{-1}) (3.4 mmol l^{-1}) were achieved in 71% of patients with established CAD. The overall result of combination gemfibrozil-lovastatin was a normalization of the lipid profile in 68% of the patients: LDL-cholesterol < 150 mg dl^{-1} (3.9 mmol l^{-1}) in all cases, triglycerides < 200 mg dl^{-1} (2.3 mmol l^{-1}) in 96% of the patients, and HDL-cholesterol > 35 mg dl^{-1} in 68% of the patients.

CONCLUSION

PPAR-alpha activators decrease triglyceride plasma levels by decreasing triglyceride synthesis and increasing triglyceride-VLDL lipolysis by increasing lipoprotein lipase gene expression and its corresponding protein synthesis and by decreasing apo C-III gene expression and its related protein synthesis; apo C-III being a natural inhibitor of lipoprotein lipase activity. Decreases in VLDL-triglyceride plasma concentrations inhibit the CETP-dependent exchange between triglycerides from VLDL and cholesteryl-esters from LDL. In this condition hepatic lipase activity is reduced on the resulting triglyceride-poor-LDL, which in turn does not reduce in size nor increase in density. Therefore, PPAR-alpha activators decrease plasma concentration in small dense-LDL which have been reported as highly atherogenic.

PPAR-alpha activators increase HDL-cholesterol plasma levels by increasing apo A-I and apo A-II gene expression and by increasing synthesis of the corresponding proteins. PPAR-alpha activators also increase HDL-cholesterol by reducing the triglyceride-VLDL mass and the resulting CETP-mediated cholesteryl-esters transfer from HDL toward VLDL. Furthermore, PPAR-alpha activators increase vascular cell expression of HDL-receptors such as ABC-1 and SRB-I and cellular cholesterol efflux. Therefore, by increasing both HDL synthesis and cellular cholesterol efflux, PPAR-alpha activators probably highly increase the 'reverse cholesterol transport' and reduce atherogensis, such as demonstrated in clinical trials showing a reduction in the progression of atheroma in atherosclerotic patients treated with bezafibrate [58–64], gemfibrozil [6, 56, 57] and fenofibrate [65].

LOCAT, VA-HIT, BECAIT and BIP studies showed that drugs belonging to the fibrate's family (gemfibrozil, bezafibrate) and acting through the stimulation of Peroxisome Proliferator Activated Receptors (PPARs) have comparable effects on plasma lipid and lipoprotein profile and on the inhibition of coronary atherosclerosis progression.

These data suggest that a simultaneous reduction in triglycerides and increase in HDL-cholesterol, without any reduction in LDL-cholesterol, in patients with low LDL-cholesterol, low HDL-cholesterol or high LDL-cholesterol, low HDL-cholesterol and high triglycerides [>200 mg dl^{-1} (2.30 mmol l^{-1})] decreases the cardiac mortality in second prevention. These studies emphasize: (1) the importance of reducing LDL-cholesterol when LDL constitute the only apo B-containing lipoprotein risk factor; (2) the additional lethal power of triglycerides in hypercholesterolaemic patients; (3) the lethal power of triglycerides in normocholesterolaemic patients with low HDL-cholesterol.

Further studies are necessary to confirm the beneficial effects of reducing triglyceridaemia and increasing HDL-cholesterol in secondary prevention and are absolutely necessary to confirm this beneficial effect in primary prevention.

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